CLAIMS

What is claimed is:

1. A method for assembling a fusion molecule, comprising:
generating a circular permutation of an insertion sequence; and
inserting the insertion sequence into an acceptor sequence.

2. The method according to claim 1, wherein the insertion sequence is inserted at a selected site in the acceptor sequence.

3. The method according to claim 1, wherein the insertion sequence is inserted at a random site in the acceptor sequence.

4. A method for assembling a modulatable fusion molecule, comprising:
generating a circular permutation of an insertion sequence;
inserting the insertion sequence into an acceptor sequence, wherein the insertion sequence and the acceptor sequence each comprise a state; and
selecting a fusion molecule, wherein the state of the insertion sequence and the state of the acceptor sequence are coupled.

5. The method according to claim 4, wherein the insertion sequence is inserted at a selected site in the acceptor sequence.

6. The method according to claim 4, wherein the insertion sequence is inserted at a random site in the acceptor sequence.

7. The method according to claim 4, wherein the state of the insertion sequence is modulated.

8. The method according to claim 4, wherein the state of the insertion sequence is modulated in response to a change in the state of the acceptor sequence.

9. The method according to claim 4, wherein the state of the acceptor sequence is modulated in response to a change in the state of the acceptor sequence.

10. The method according to claim 4, wherein the state of the acceptor sequence is modulated in response to a change in the state of the insertion sequence.

11. The method according to claim 4, wherein the fusion molecule comprises a new state.

12. A method for assembling a multistable fusion molecule which can switch between at least an active state and a less active state, comprising:

circularly permuting an insertion sequence;

inserting the insertion sequence into an acceptor sequence, thereby generating a fusion molecule, wherein either the insertion sequence or the acceptor sequence comprises a state; and wherein the respective other sequence is responsive to a signal; and

selecting a fusion molecule, wherein the state is coupled to the signal, such that the fusion molecule switches state in response to the signal.

13. The method according to any of claims 1, 4, and 12 wherein said insertion sequence and said acceptor sequences comprise nucleic acids.

14. The method according to claim 13, comprising:

obtaining a first nucleic acid fragment comprising an insertion sequence and a second nucleic acid fragment comprising an acceptor sequence and inserting said first nucleic acid fragment into said second nucleic acid fragment.

15. The method according to claim 14, further comprising providing a library of fusion nucleic acids encoding fusion polypeptides, said fusion nucleic acids comprising insertion sequences inserted into acceptor sequences, and selecting fusion polypeptides wherein the states of the insertion and acceptor polypeptides are coupled.

16. A method for modulating a cellular activity, comprising:

providing a fusion molecule generated according to the method of any of claims 1, 4, 12 and 15 to a cell, wherein a change in state of at least the insertion sequence or the acceptor sequence modulates a cellular activity, and wherein the change in state which modulates the cellular activity is coupled to a change in state of the respective other portion of the fusion molecule; and

changing the state of the respective other portion of the fusion molecule, thereby modulating the cellular activity.

17. A method for delivering a bio-effective molecule to a cell, comprising:

providing a fusion molecule associated with a bio-effective molecule generated according to the method of any of claims 1, 4, 12 and 15 to the cell, the fusion molecule comprising an insertion sequence and an acceptor sequence, wherein either the insertion sequence or the acceptor sequence binds to a cellular marker of a pathological condition and wherein upon binding to the marker, the fusion molecule dissociates from the bio-effective molecule, thereby delivering the molecule to the cell.

18. A method for delivering a bio-effective molecule intracellularly, comprising:

providing a fusion molecule associated with a bio-effective molecule generated according to the method of any of claims 1, 4, 12 and 15 to the cell, the fusion molecule comprising an insertion sequence and an acceptor sequence, wherein either the insertion sequence or acceptor sequence comprises a transport sequence for transporting the fusion molecule intracellularly, and

wherein release of the bio-effective molecule from the fusion molecule is coupled to transport of the fusion molecule intracellularly.

19. The method according to claim 18, wherein either the insertion sequence or the acceptor sequence is capable of binding to a biomolecule, and

wherein binding of the fusion molecule with the biomolecule localizes the fusion molecule comprising the bio-effective molecule intracellularly and disassociates the bio-effective molecule from the fusion molecule.

20. A method for modulating a molecular pathway in a cell, comprising:

providing a fusion molecule generated according to the method of any of claims 1, 4, 12 and 15 to the cell, the fusion molecule comprising an insertion sequence and an acceptor sequence,

wherein the activity of the insertion sequence and acceptor sequence are coupled, and responsive to a signal, and

wherein the activity of either the insertion sequence or the acceptor sequence modulates the activity or expression of a molecular pathway molecule in the cell; and exposing the fusion molecule to the signal.

21. A method for controlling the activity of a nucleic acid regulatory sequence, comprising:

providing a fusion molecule generated according to the method of any of claims 1, 4, 12 and 15, the fusion molecule comprising an insertion sequence and an acceptor sequence,

wherein either the insertion sequence or the acceptor sequence responds to a signal, and

wherein the respective other sequence of the fusion molecule binds to the nucleic acid regulatory sequence when the signal is responded to; and

exposing the fusion molecule to the signal.

22. A sensor molecule for detecting a target analyte, comprising:

an insertion sequence and an acceptor sequence, generated according to the method of any of claims 1, 4, 12 and 15,

wherein either the insertion sequence "pr" the acceptor sequence binds the analyte, and wherein binding of the analyte is coupled to production of a signal from the sensor molecule.

23. A fusion molecule, comprising:

an insertion sequence and an acceptor sequence, generated according to the method of any of claims 1, 4, 12 and 15,

wherein either the insertion sequence or the acceptor sequence transports the fusion molecule intracellularly and wherein intracellular transport of the fusion molecule is coupled to binding of the fusion molecule to a bio-effective molecule.

24. A fusion molecule, comprising:

an insertion sequence and an acceptor sequence generated according to the method of any of claims 1, 4, 12 and 15, wherein either the insertion sequence or the acceptor sequence binds to a nucleic acid molecule, and wherein nucleic acid binding activity is coupled to the response of the respective other sequence of the fusion molecule to a signal.

25. A fusion molecule, comprising:

an insertion sequence and an acceptor sequence generated according to the method of any of claims 1, 4, 12, and 15,

wherein either the insertion sequence or the acceptor sequence associates with a bio-effective molecule, and disassociates from the bio-effective molecule, when the respective other sequence of the fusion molecule binds to a cellular marker of a pathological condition.

26. A fusion molecule capable of switching from a non-toxic to a toxic state, comprising:

an insertion sequence and an acceptor sequence generated according to the method of any of claims 1, 4, 12 and 15,

wherein either the insertion sequence or the acceptor sequence binds to a cellular marker of a pathology, and

wherein binding of the marker to the fusion protein switches the fusion protein from a non-toxic state to a toxic state.

27. A fusion molecule capable of switching from a toxic state to a less toxic state, comprising:

an insertion sequence and an acceptor sequence generated according to the method of any of claims 1, 4, 12 and 15, wherein either the insertion sequence or acceptor sequence binds to a cellular marker of a healthy cell, and

wherein binding of the marker to the fusion protein switches the fusion protein from a toxic state to a less toxic state.

28. A molecular switch for controlling a cellular pathway, comprising:

a fusion molecule comprising an insertion sequence and an acceptor sequence generated according to the method of any of claims 1, 4, 12, and 15,
wherein the states of the insertion and acceptor sequences are coupled, and responsive to a signal, and

wherein the state of either the insertion sequence or the acceptor sequence modulates the activity or expression of a molecular pathway molecule in a cell.

29. A modified molecular switch generated according to the method of any of claims 1, 4, 12, and 15, wherein said molecular switch is responsive to at least one ligand that differs from a ligand recognized by the unmodified form of said switch.

30. A library, comprising a plurality of library members,

wherein each library member comprises a first nucleic acid sequence encoding a first polypeptide having a first state, the first nucleic acid sequence having been circularly permuted and inserted into a second nucleic acid sequence encoding a second polypeptide having a second state.

31. A library comprising a plurality of library members comprising fusion molecules generated according to any of claims 1, 4, and 12.

32. A library generated according to claim 31, wherein said library is generated by iterative processing of at least one library generated according to any of claims 1, 4, and 12.

33. A library according to claim 32, generated by inserting a selected circularly permuted insert sequence generated from a first library into an acceptor sequence, to generate a second library having a plurality of members each comprising said selected circularly permuted insert sequence.

34. A library according to claim 33, wherein said selected circularly permuted insert sequence is inserted at a random site in the acceptor sequence.

35. A library according to claim 33, wherein said selected circularly permuted insert sequence is inserted at a non-random site in the acceptor sequence.

36. An isolated nucleic acid encoding a molecular switch protein comprising a nucleotide sequence selected from any of SEQ ID NOS: 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74, or an effective fragment thereof.

37. A molecular switch protein comprising an amino acid sequence selected from any of SEQ ID NOS: 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, and 75, or an effective fragment thereof.

38. A method for assembling a fusion molecule, comprising:
generating a random circular permutation of an insertion sequence; and
inserting the insertion sequence into an acceptor sequence.

39. The method according to claim 38, wherein the insertion sequence is inserted at a selected site in the acceptor sequence.

40. The method according to claim 38, wherein the insertion sequence is inserted at a random site in the acceptor sequence.

41. A method for assembling a modulatable fusion molecule, comprising:
generating a random circular permutation of an insertion sequence;
inserting the insertion sequence into an acceptor sequence, wherein the insertion sequence and the acceptor sequence each comprise a state; and
selecting a fusion molecule, wherein the state of the insertion sequence and the state of the acceptor sequence are coupled.

42. The method according to claim 41, wherein the insertion sequence is inserted at a selected site in the acceptor sequence.

43. The method according to claim 41, wherein the insertion sequence is inserted at a random site in the acceptor sequence.

44. The method according to claim 41, wherein the state of the insertion sequence is modulated.

45. The method according to claim 41, wherein the state of the insertion sequence is modulated in response to a change in the state of the acceptor sequence.

46. The method according to claim 41, wherein the state of the acceptor sequence is modulated in response to a change in the state of the acceptor sequence.

47. The method according to claim 4, wherein the state of the acceptor sequence is modulated in response to a change in the state of the insertion sequence.

48. The method according to claim 41, wherein the fusion molecule comprises a new state.

49. A method for assembling a multistable fusion molecule which can switch between at least an active state and a less active state, comprising:

randomly circularly permuting an insertion sequence;

inserting the insertion sequence into an acceptor sequence, thereby generating a fusion molecule, wherein either the insertion sequence or the acceptor sequence comprises a state; and wherein the respective other sequence is responsive to a signal; and

selecting a fusion molecule, wherein the state is coupled to the signal, such that the fusion molecule switches state in response to the signal.